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It has been proposed that membrane protein pump function is modulated by the lipid monolayer spontaneous curvature, an elastic parameter of the monolayers related to the tendency for the lipids to form non-bilayer phases. [Gruner, Proc. Natl. Acad. Sci., USA 82 (1985) 3665]. To test this hypothesis Ca^{2+} ATPase from rabbit sarcoplasmic reticulum has been reconstituted into vesicles of different lipids with well characterized values of the spontaneous curvature. The activity is found to correlate with the spontaneous curvature and not with the specific lipids used to achieve a given value of spontaneous curvature. Progress is also reported on the development of alternative methods of measuring spontaneous membrane curvature.

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Date: 28 June 1988

Progress Report on Contract N00014-86-K-0396-P00001

Principal Investigator: Dr. Sol M. Gruner

Contractor: Princeton University

Contract Title: Lipid Dependent Mechanisms of Protein Pump Activity

INTRODUCTION:

The physical parameter of lipid bilayers which modulate the activity of integral protein membrane are not well understood. It has been proposed that the value of an elastic parameter of the lipid monolayers, known as the monolayer spontaneous radius of curvature, R_0, is important to the activity of certain membrane proteins [1]. The overall goal of this research is to test this hypothesis. This involves three objectives:

1) Develop methods of measuring the spontaneous radius of curvature of lipid membranes and better understand the physical effects of varying R_0.
2) Correlate the activity of a well characterized pump protein with the R_0 value of the imbedding bilayer. If R_0 is important, then the pump activity should correlate with R_0, not with the specific lipid mix used to achieve a given R_0 value.
3) Determine if bacterial membranes act to keep R_0 fixed when the bacteris are forced to alter their membrane lipid composition.

PROGRESS:

Objective 1). A method of measuring R_0 for pure lipids (e.g., in the absence of protein has been developed [2,3]. However, it is desirable to develop methods which operate in bilayers containing protein and over a wider range of R_0 values. There is evidence that the ^{2}H-NMR quadrople splitting, ΔQ of deuterated lipid chains may correlate with R_0 [4]. Toward this end, the use of perdueterated compounds as R_0 probes was investigated. Relatively inexpensive lipid analogs, such as perdeuterated alkanols were found to strongly perturb membranes at levels required for good NMR signals. In consequence, perdeuterated phospholipid was recently synthesized. We are just now beginning to correlate the R_0

values measured by the x-ray technique to ΔQ. This work is being done in collaboration with Drs. P. Cullis and C. Tilcock of the University of British Columbia.

Work has also been performed on understanding the physics of R_0 variation in membranes. An apparatus has been constructed which allows x-ray diffrction studies of lipid suspensions at pressures of up to 1000 bar. As expected, R_0 appears to be very pressure sensitive. A note describing the pressure effects will be submitted for publication in the near future.

Objective 2). In collaboration with Drs. A. Janoff and M. Jaworsky of the Liposome Co., Ca^{++} ATPase from rabbit sarcoplasmic reticulum has been reconstituted in vesicles of binary mixtures of the lipids DOPE, DOPE-Me and DOPC. For each mixture, the pump activity (moles Ca^{+2} pumped/moles ATP hydrolyzed) and the R_0 value of the binary lipid mixture were measured. It was found that the activity correlated simple with the measured R_0 values, that is to say, when pump activity was graphed against R_0, all points fell on a simple smooth curve. Different binary mixtures which had the same R_0 value yielded similar pump activities. Control experiments are being done to see if the correlation is due to systematic variation in the vesicle sizes with composition. A publication describing these results will soon be prepared.

Objective 3). Lipid extracts of bacteria grown under conditions which cause the lipid composition to vary were examined to see if R_0 was being held constant. Unfortunately, we have been only marginally successful in measuring R_0 values on these lipids because the R_0 values appear to be outside the range of our present techniques (whence, objective #1). In consequence, we are switching to another strain of bacteria which, we expect, will yield small R_0 values. These cultures are being grown now. These experiments are done in collaboration with Dr. R. McElhaney of the University of Alberta and Dr. G. Lindblom of the University of Umea, Sweden.

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[1] S.M. Gruner, Proc. Natl. Acad. Sci. *82* (1985) 3665.

[2] S.M. Gruner, V.A. Parsegian and R.P. Rand, Faraday Discuss. Chem. Soc. *81* (1986) 29.

[3] S.M. Gruner, M.W. Tate, G.L. Kirk, P.T.C. So, D.C. Turner, D.T. Keane, C.P.S. Tilcock and P.R. Cullis, Biochem. *27* (1988) 2853.

[4] P.R. Cullis, M.J. Hope and C.P.S. Tilcock, Chem. Phys. Lipids *40* (1986) 127.

WORK PLAN:

1) Sufficient quantities of perdeuterated lipid are in hand. We intend to proceed immediately to correlate the ^{2}H-NMR of these materials with R_0 values derived by x-ray diffraction.
2) The Ca^{++} ATPase will next be reconstituted in a broader range of lipids. Systematic control studies on the vesicle sizes will also be done to insure that the observed correlation is not simply a vesicle size effect. It is also known that R_0 is a sensitive function of pressure. We will map out how R_0 changes with pressure for representative lipid mixtures. It would be of interest to see if the correlation of pump activity varies with pressure as expected from the variation in R_0 due to composition.
3) We hope to have enough lipid extracts from new bacterial cultures by late summer to proceed with R_0 measurements. We will first use the x-ray diffraction measurement procedure. If difficulties are encountered with this, we will have to wait until the NMR method is refined.
4) Investigation of the effects of lipid spontaneous curvature will be extended to oligopeptide membrane channels, as discussed in the continuation application for this contract.

INVENTIONS: None

PUBLICATIONS AND REPORTS (YEARS 1-2)

1) E. Shyamsunder, S.M. Gruner, M.W. Tate, D.C. Turner, P.T.C. So and C.P.S. Tilcock (1988). Observation of inverted cubic phase in hydrated dioleoylphosphatidylethanolamine membranes, *Biochem. 27*: 2332-2336.
2) E. Shyamsunder and S.M. Gruner. An x-ray diffraction study of the effects of pressure on H_{II} phase lipid. (Manuscript in preparation.)

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